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THE SEAT OF SMELL IN THE CRAYFISH.

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The location of the sense of smell in the crustacea has been the subject of but few investigations. Putnam, Graber, Bateson, Dearborn, Spaulding and others have made observations on the reactions of various decapods to food at a distance from the body, although they were not primarily concerned in tracing the seat of the olfactory reactions. Herrick, working on the lobster, found that most parts of the body were sensitive to ammonia and clam juice. Experiments of Nagel [1] and Bethe [2] indicate that, in the decapods, the first antennæ have an important olfactory function, although both these investigators regard the olfactory sense organs as not exclusively located in these appendages. Nagel worked with *Pagurus*, *Astacus* and *Carcinus*. Removal of the antennules of *Pagurus* was followed by reactions to chemical stimuli, although these were not so pronounced as before the operation. *Astacus* in the presence of food was found to show lively movements of the antennules, followed by movements of the mouth parts and legs. *Carcinus* showed the same movements of the mouth parts and antennæ in the presence of food, but it was only when food came into actual contact with the mouth parts that efforts were made to secure it. Nagel's experiments led him to conclude that sight and touch play a greater part in locating food than the sense of smell.

Bethe found that if a piece of meat is thrown into a dish with several *Carcinus* there is no reaction at first. After several seconds the antennules begin to wave rapidly and the maxillipeds move slowly back and forth. The animals nearest the piece of meat

[1] Bibliotheca Zoologica, Bd. 18, 1894.
[2] *Archiv f. mik. Anat.*, Bd. 50, 1897.

give this reaction first. The farther the *Carcinus* is from the meat the later will begin the reactions. Soon after the animals begin to react they move about and usually in quite a direct line toward the meat. The nearer they approach the food the faster they go. That the eye plays little part is evidenced by the fact that blinded forms go toward the meat with just as much certainty. If a piece of meat is passed over the rocks at the bottom of the aquarium a *Carcinus* will follow this scent and often pick up every stone, which has been in contact with the meat, and pass it to the mouth, but as soon as the stones come in contact with the maxillipeds they are rejected.

If the antennules are removed *Carcinus* reacts to food but only when it is placed a short distance from the mouth parts. The latter are regarded by Bethe as also sensitive to chemical stimuli. In the amphipod *Amphithoe*[1] one of the present writers found that the antennules were important olfactory organs, but that there remained a certain but much diminished power to detect food at a distance from the mouth parts after the antennules, or even the antennules and the antennæ were removed. Other species of amphipods were found to make exploring movements of the antennules when in the presence of food.

Nagel's experiments with amphipods and isopods failed to elicit any positive response, although some species gave negative responses to disagreeable or irritating substances. The terrestrial isopods, *Oniscus*, *Porcellio* and *Armadillo* he found to be quite insensitive to strong substances like cedar oil and benzol. It is not difficult, however, to obtain very decided reactions from terrestrial isopods to the vapor of acetic acid and ammonia even after removal of the antennæ.

Many papers have appeared dealing with the organs of smell in crustacea but conclusions regarding their functions have been based more on morphological grounds than on the results of experiment. One of the most recent papers on the chemical sense of crustacea is that of Bell,[2] who worked on the reactions of the crayfish. Bell applied meat juice by means of a fine-pointed pipette, to various parts of the body. To render it evi-

[1] BIOLOGICAL BULLETIN, Vol. 2, p. 165, 1901.

[2] *Jour. Comp. Neur. and Psych.*, 16, 299, 1906.

dent when the substance came in contact with the animal, it was colored with eosin or carmine. It was found that stimulation of various parts of the body would elicit a response. The animal moved its mouth parts, became restless, moved the small chelipeds about and sooner or later attempted to go toward the source of stimulus. The responses varied somewhat according to the region to which the stimulus was applied, but there was a marked tendency, as we have also found, for the first reaction to manifest itself by a movement of the part most directly stimulated. The antennules, antennæ, mouth parts and tips of the large and small chelipeds were found to be organs of especial sensitiveness. We have repeated Bell's experiments with meat juice and verified in the main his results. Bell, however, did not test his conclusions by removal of certain organs or by operations on the nervous system. One is never quite sure that by using chemicals which diffuse through the water only the organ is stimulated to which the stimulus is applied. It was thought desirable, not only for this reason, but on account of determining more definitely the role of certain organs in chemoreception to observe the reactions of crayfish after the removal of the antennules and antennæ, and after the destruction of the brain or the division of the nerve cord.

The outer ramus of the antennules bears, in addition to the kinds of setæ found on the antennæ and other parts of the body, certain peculiar club-shaped organs which have generally been considered the end organs of the sense of smell. These organs are absent on the inner ramus, and on the antennæ as well as other parts of the body. Organs of a similar character are common in other crustacea, being in many cases more abundantly developed in the male. This circumstance, as well as the fact that removal of the antennules diminishes the response to olfactory stimuli, renders it quite probable that these club-shaped organs perform the function which has so often been assigned to them. In a series of experiments we have compared the reactions of crayfish with the outer ramus of the antennules removed with the reactions of normal animals and individuals otherwise operated on. The rami were removed several days before the animals were experimented with in order to eliminate any effect of shock

on the reactions. All of the specimens were kept for the same period without food, were placed in the same kind of dishes and treated in all respects as nearly as possible in the same way. Only individuals which had been resting quietly for some minutes were employed for experiment. If any reaction was produced by the movements of the observer the crayfish was not used for experiment until it had been left for a considerable time and became perfectly quiet. A piece of meat was very carefully introduced into the water with a long fine forceps and held a quarter of an inch above the rostrum. If one's movements are exceedingly slow it is possible to work around crayfish even when recently brought in from the field without exciting alarm. The number of seconds was noted before any of the feeding responses of the legs or mouth parts were set up. Sometimes such movements appear to arise spontaneously, and therefore only those cases were counted in which these movements eventuated in seizing and devouring the meat. In three sets of experiments performed at different times the time reactions of the crayfish were as follows:

EXPERIMENT I.

	Time of Reactions in Seconds.								Average.
Outer ramus removed	60	80	42	16	36	220	64		74
Inner ramus removed	80	34	64	74	30	6			48
Both rami removed	110	170	270	88	180	156	58	116	131
Normal animal	46	12	6	30	36	68	30	24	31

EXPERIMENT II.

						Average.
Outer ramus removed	184	148	92	272		174
Inner ramus removed	36	20	24			27
Both rami removed	50	64	120	90		81
Normal animal	2	18	6			8.7

EXPERIMENT III.

							Average.
Outer ramus removed	10	24	26	24	44		25.6
Inner ramus removed	10	18	24	42			23
Both rami removed	112	120	412	26			167.5
Normal animal	26	16	26				22.7

EXPERIMENT IV.

(Specimens with eyes blackened over.)

Outer ramus removed	72	116	180	120	36	94	30	110	144				100
Inner ramus removed	6	12	4	4	4	6	36	12	4	48	14		13.7
Both rami removed		42	112	242	270								141.5

EXPERIMENT V.

(Specimens with eyes blackened over.)

Outer ramus removed	74	68	214	56	168						118
Inner ramus removed	10	42	14	32	102	32	34	27	6	33	33.2

The experiments show in a striking enough way that removal of the outer branch of the antennules is followed by a much greater loss of sensitiveness to olfactory stimuli than is caused by removal of the inner ramus. But they also show that crayfish with the inner ramus removed react less promptly than do normal animals. We may conclude from these experiments that the part most sensitive to olfactory stimuli is the outer ramus of the antennules, but that the inner ramus is also to a certain extent sensitive to the same kind of stimuli. Specimens with both rami removed respond to meat juice or pieces of meat placed near the tip of the large second antennæ. It is scarcely possible that any chemical could diffuse to other parts of the body before the reaction takes place and hence the second antennæ must have an olfactory function also. Specimens in which both antennules and antennæ were removed showed a marked power of responding to bits of meat or meat juice placed near the mouth parts or the tips of the chelæ. The small chelipeds were found especially sensitive to substances placed near the tip. By very carefully applying the tip of a pipette which had been drawn out into a long slender tube near one of the small chelæ and slowly forcing out a little meat juice, one usually sees a slight grasping movement of the chelæ, often at first a small twitch of the dactyl, followed by movements of reaching about. These movements are followed by exploring movements of the other chelipeds, chewing movements of the mouth parts, and by a turning of the body toward the stimulus.

Animals in which the brain was destroyed were also experimented with. The crayfish was securely fastened to facilitate working upon it. With a fine-pointed scalpel a piece of the carapace about one fourth of an inch square was cut out just over the brain. After destroying the brain the piece of the carapace was carefully replaced and the edges sealed with asphalt varnish. The animal was then kept out of water until the asphalt had dried and the wound sealed. After a few days the animal was experi-

mented with and showed marked responses from the mouth parts and chelipeds. When the chelipeds were rubbed with a piece of filter paper the substance was seized but rejected if passed to the mouth parts. When meat was pressed against the chelipeds in the same way it was seized more eagerly, produced a greater degree of excitement and was generally eaten. To make sure that this was not due to a difference in the consistency of the two articles two wads of filter paper, were taken upon one of which was pressed some meat juice. The two wads were applied to the chelipeds in the same way, but the one with the meat juice was more quickly seized and was generally passed to the mouth parts and swallowed. In some instances the pure filter paper was swallowed, but this was very rare.

The chelipeds were also stimulated simultaneously, the one with pure filter paper, the other with filter paper soaked in meat juice. The animal almost invariably turned toward the side with the meat juice and grasped more vigorously on that side. The stimuli were frequently transposed but this did not affect the result; the reaction to the paper with the meat juice was the more vigorous.

In one crayfish the nerve cord was cut across directly behind the mouth parts and in front of the large chelipeds. The result of this operation is to somewhat paralyze the animal, especially the anterior chelipeds which are drawn up under the body. Objects that touch the chelæ are frequently passed to the mouth but again rejected. A bit of meat, however, is chewed and swallowed although this act requires a much longer time than in the normal animal. In some cases the chewing movements ceased before the food was swallowed. The chelipeds when stimulated by meat grasp it more eagerly than they seize innutritious materials, and the animal in general shows a greater degree of excitement.

The experiments recorded show that the outer ramus of the antennules which bear the so-called olfactory setæ are especially sensitive to olfactory stimuli; that the inner ramus of the antennules and the antennæ are also sensitive to olfactory stimuli but to a less degree; and that the olfactory sense is developed in other parts of the body, especially the mouth parts and tips of the chelipeds.

ANALYSIS OF FORM REGULATION WITH THE AID OF ANÆSTHETICS.

C. M. CHILD.

During the last year and a half the writer has carried on an extensive series of experiments on the influence of certain anæsthetics on the course and results of form regulation in *Planaria dorotocephala* Woodworth. Since the method has proved to be of considerable value and since it will be some time before the results of these and other experiments planned for the future can be presented in full, it seems desirable to present briefly and without extended comment the most important features of the work up to date as an illustration of the value of the method and the results to be attained by it.

In my experiments thus far I have used chiefly alcohol, ether and acetone-chloroform, known commercially as chloretone. Although alcohol is much less strongly anæsthetic than many other substances its action is in general similar in kind to that of the substances commonly known as anæsthetics. The results with the three substances mentioned are practically identical so far as the more general features are concerned, though various differences of detail appear, which need not be considered here. Incidentally, however, it may be noted that these differences in the effects of the different anæsthetics serve to some extent as a means of determining the specific differences in the effects of different anæsthetics on the metabolic processes: how far they will prove to be of value in this direction only the future can determine.

In my experiments various concentrations of the three substances have been employed, but the most characteristic results in general have been obtained with alcohol 1.5 per cent., ether 0.4–0.5 per cent., chloretone 0.025–0.0375 per cent. These concentrations cause a considerable deathrate, but the results in the pieces which do not die within the first week or ten days are very uniform, so far as pieces of the same size and from the same region of the body are concerned. Alcohol has been used to a

larger extent than either chloretone or ether in my experiments up to the present time because it has given characteristic results and because it has seemed desirable to obtain fairly complete data concerning the effect of a mild anæsthetic upon various regions of the body, on pieces of different size and age and in different physiological condition as a basis for comparative work with substances with stronger anæsthetic properties. It is my intention to extend the work to various other groups of substances which have a definite effect on metabolism. In most of the work of this kind the effect of only a short period of time in the given medium has been determined. It seems to me particularly desirable to determine more exactly the effects of continued existence in media of various constitution on development and regulation. By such means it should be possible to advance a step further in our analysis of the processes of differentiation, localization and growth.

I. Methods.

The method of use of the anæsthetics, all three of which are more or less volatile, is as follows: Not more than ten pieces of the planarian are placed in a Stender dish holding about 250 c.c. and with ground edge and cover with ground groove exactly fitting the edge. This dish is filled to three fourths of its depth with the mixture of the desired concentration, some of the fluid is poured over the under side of the cover so as to wet its surface and fill the groove, and the cover is placed in position at once. Several of these dishes are placed in larger glass jars with ground edges, a liter or more of the fluid is poured over them into the jar and it is at once sealed by means of a glass plate on which a ring of vaseline corresponding to the edge of the jar has been smeared. In this way decrease in concentration is avoided as far as possible. Furthermore the fluid is renewed at least every forty-eight hours and the mixture of the desired concentration is always made up immediately before using. It has been found desirable to keep the temperature as nearly as possible constant, except where its influence on the results was to be determined. In most of my experiments the temperature has ranged between 17° and 20° C. but rarely beyond these limits. Rise in temperature much beyond 20° C. increases the

death-rate and in temperatures below 15° C. the regulatory processes are considerably retarded and certain complicating features which result directly from low temperature appear. With fairly constant temperature and regular renewal of the fluid it has been possible to keep various individuals and pieces alive in 1.5 per cent. alcohol for several months, and death, when it occurred, was apparently the result of starvation. As will appear below, however, the length of life of the pieces and individuals varies greatly according to conditions.

II. Certain Features of the Organization of Planaria.

In order to make clear certain of the results presented below it is necessary to call attention to the fact that in *Planaria dorotocephala* (and in *P. maculata*) the posterior region of the body in all animals above a certain small size is specified to a greater or less extent as a second individual or zoöid, which, when it attains a certain degree of physiological independence, separates from the other part by an independent motor reaction and becomes a complete animal. As I pointed out some years ago,[1] the presence of this second zoöid is indicated by the regional differences in the regulatory power of short pieces taken in sequence. When we compare pieces not more than one tenth the length of the whole animal and as nearly as possible of equal length, we find that the ability to form a head decreases in each successive piece from the original head-region posteriorly, until at about the region of the old pharynx the power to form a head disappears entirely and the pieces remain headless. But at about the middle of the post-pharyngeal region, *i. e.*, at the level where fission occurs, the power of head-formation reappears rather suddenly. In species like *Planaria simplicissima*, which do not undergo fission, there is no increase in the power of head-formation in the postpharyngeal region. Other regional differences which indicate the presence of a second zoöid in the posterior region of the body are described in the paper referred to above. As further evidence along the same line, I may add that I have been able to induce fission experimentally without feeding under conditions where it does

[1] Child, "The Relation between Regulation and Fission in *Planaria*," Biol. Bull., XI., 3, 1906.

not occur normally, *e. g.*, in animals reduced by starvation from a length of 15–18 mm. to a length of 6–7 mm., in young animals of 7–8 mm. in length, in the pieces resulting from the fission of large individuals, and again in the pieces resulting from the fission of pieces derived from fission.

It follows from these facts that the posterior end of the first zoöid lies somewhere near the middle of the postpharyngeal region in large individuals, and posterior to this level is a second young individual.

III. The General Effects of the Anæsthetics.

In the first place the regulatory processes are much retarded and the retarding effects are greater in small than in large pieces. The most favorable cases in the anæsthetics require at ordinary temperatures ten days or more to reach a stage which similar pieces in water attain in four or five days.

In a short paper[1] attention has already been called to the fact that the change in the proportions of the pieces is retarded to a much greater extent than the formation of new parts. As a matter of fact the increase in length and the decrease in width of the pieces, which is so characteristic a feature of the regulation in *Planaria* does not occur to any appreciable extent until the pieces have become sufficiently acclimated and have reached a sufficiently advanced stage of development to move about in a manner approaching the normal. In the paper above referred to I pointed out that this fact confirms the conclusions which I have already stated in previous papers, viz., that this change in proportion is largely the result of mechanical conditions connected with movement and locomotion.

In general the formation of the new tail is retarded to a much greater extent than that of the head. A small amount of new tissue forms on the posterior cut surface, but outgrowth to form the typical, tapering posterior end does not occur until the specimens begin to creep about. Apparently the movement and use of the posterior end is not only a factor in determining its shape, but also stimulates the growth of new tissue.

[1] Child, "The Regulatory Change of Shape in *Planaria dorotocephala*," Biol. Bull., XVI., 6, 1909.

The process of regulation ceases in the anæsthetic before the degree of development attained in water is reached. The head remains of smaller size in proportion to the size of the piece, the "auricles" on the sides of the head, which in this species are normally long and pointed, remain short and blunt, the new pharynx attains only a fraction of the size attained in water, and intestinal regulation, with the exception of the degeneration of the smaller intestinal branches in many cases, does not occur to any appreciable extent until the animals begin to move about actively.

These results are sufficient to show that the method affords a means of separating more or less completely in time certain regulatory processes which under normal conditions occur together, and thus of determining in some degree their relations to each other, and in the cases of change of shape and development of the tail their relation to movement and use of parts.

Another most interesting difference between the pieces in anæsthetics and those in water is the relation between regeneration in the stricter sense and redifferentiation. In certain pieces, which in water form the whole head back as far as the eyes by regeneration of new tissue, only the more anterior portions of the head are formed in this way in anæsthetics. In this respect alcohol and ether differ to some extent: in ether apparently less regeneration and more redifferentiation than in alcohol occurs, and in some cases in ether only the extreme tip of the head is regenerated, all other parts being the result of redifferentiation. A case of this kind is figured in my paper referred to above (9, Figs. 14–16). As regards this point the process of regulation resembles that occurring after long continued starvation, but the substitution of redifferentiation for regeneration is even more complete in the anæsthetics than after starvation.

IV. The Effect in Relation to the Size of the Piece.

In general a larger piece is necessary for the production of a whole, or anything approaching a whole, than in water. Within certain limits the death-rate increases as the size of the piece decreases. Regulation is retarded to a much greater extent in smaller than in larger pieces. In smaller pieces the formation

of a single median eye-spot instead of two, bilaterally symmetrical in position, is much more frequent than in larger pieces. With decrease in size all stages between the fully developed head — *i. e.*, as fully developed as it ever becomes in an æsthetic media — and headless pieces can be obtained.

All of these differences in regulation which are connected with differences in size can be observed in water, but in the anæsthetics they occur in much larger pieces than in water.

V. The Effect in Relation to Region of the Body.

The regional differences are similar in character to those observed in water but some of them are more marked. In the anæsthetics, for example, pieces from the middle region of the body, even those as long as one fourth of the whole length, almost always remain headless, while in water only very short pieces from the middle region fail to produce heads. Pieces one fourth the length of the body taken from the region immediately behind the old head almost always produce heads in the anæsthetic as well as in water. In short, it is almost impossible to inhibit head-formation in pieces from this region, though there is not the slightest difficulty in inhibiting it completely in pieces of the same size from the middle region. When we compare pieces of the same size taken in sequence from the anterior end posteriorly, we find that the ability to form a head disappears much more rapidly in anæsthetics than in water, but at the region of fission it appears again in both media and the pieces from the posterior region of the body, *i. e.*, the pieces of the second zoöid, so far as they survive — they are like pieces of younger animals in being more sensitive than pieces from other regions when first placed in the anæsthetic, and in becoming more rapidly and completely acclimated — produce a larger percentage of normal heads (that is, normal for the medium) than pieces from any other region.

Manifestly the comparison of pieces from different regions shows much more clearly than any series of experiments in water that the ability to form certain parts is present in very different degree in different regions of the body, and since the effect of the anæsthetics is to depress the metabolic processes or certain of them, the ability to form certain parts is apparently closely connected with the intensity of certain metabolic processes.

VI. The Effect on Localization and Number of Parts.

The localization of the pharynx in anæsthetics is different from its localization in water. In prepharyngeal pieces the pharynx appears nearer the posterior end and in postpharyngeal pieces, nearer the anterior end than in water. This difference in the localization of the pharynx means that the process of redifferentiation does not extend so far from the posterior end in prephargeal pieces, or from the anterior end in postpharyngeal pieces as in water. Moreover, the shifting of the pharynx toward the middle, which occurs in each piece in water, is greatly retarded and in many cases never completed in the anæsthetics.

This change in the localization of a specific organ resulting from a change in the external medium is of considerable interest and has a bearing upon the problem of localization in general. Driesch has maintained that the localization of morphogenetic processes in "harmonious equipotential systems," of which he regards *Planaria* as an example, cannot be accounted for on a physico-chemical basis. In this case the facts certainly demonstrate that a change in the constitution of the medium, of such a character as to affect the metabolic processes, brings about a change in the localization of a specific organ, the pharynx.

Another case which involves not merely localization but number of parts as well, concerns the eye-spots. Normally two eye-spots, symmetrically placed, appear in *Planaria* in regulation as well as in ontogeny. As noted above, however, we find that short pieces in water, especially those from the middle region of the body, often give rise to only one median eye-spot. In fact the formation of a single eye-spot instead of two is the first indication of decreased or incomplete ability to form a head. If we compare pieces of different lengths with anterior ends at the same level of the body we find that with decreasing length of piece single eye-spots appear more frequently, until in pieces of a certain length perhaps only single eye-spots are formed. With still further decrease in the length of the pieces the heads with single eye-spots give way to a headless condition. It should be noted, however, that the relation between the length of the piece and the character of the result is by no means constant, but is dependent on the length of the animal, the physiological condition

and various other factors. In shorter individuals, for example, a relatively larger fraction, though an absolutely shorter piece, is necessary for a certain result similar to that produced by a relatively smaller fraction, though absolutely longer piece from a longer individual. Moreover, even in the same individual paired eyes appear in pieces of a certain length from the anterior region and the region of the second zoöid, while pieces of the same length from the middle region produce either single eyes or else remain headless. With continued starvation the length of a piece necessary to produce a given result as regards eyes increases.

With this brief statement of the results of experiments in water we may now turn to the consideration of the anæsthetics. In the anæsthetics single eyes appear in much longer pieces than in water. By preparing similar pieces of the proper length from a certain region of the body and placing part of them in alcohol, or ether, part in water, it is often possible to obtain 100 per cent. of single eyes in the anæsthetic medium and 100 per cent. of normal paired eyes in the water.

But in the anæsthetic a rather remarkable further change occurs very frequently, though I have never observed it with certainty in water. After some two weeks or more in the anæsthetic the single-eyed heads give rise to two additional eyes symmetrically placed in the normal position and slightly posterior to the single median eye already present. These three-eyed individuals are a characteristic feature of the experiments with anæsthetics. All the eye-spots persist as long as the animals live, whether they remain in the anæsthetic or are returned to water.

But still another feature must be noted. The longer pieces in the anæsthetic frequently produce two eyes in the normal position. In many cases such pieces give rise after two weeks or more to a second pair of eye-spots a short distance behind the first pair and, like them, symmetrically placed. Such individuals then possess four eye-spots, all of which persist during life.

Briefly then a very common effect of the anæsthetic is to increase the number of the eye-spots beyond the normal. It should perhaps be noted that these additional eye-spots are not mere fragments of pigment, but typical eyes, the pigment being

associated with the unpigmented area representing the sensory cells in each case. Frequently, however, in the four-eyed individuals the unpigmented areas as seen in dorsal view in the living animals are continuous between the anterior and posterior eye on each side. But the pigment spots are, so far as my observations go, always distinct and arise separately, not by division of a pigment mass already present. An attempt to consider the possible factors involved in these peculiar phenomena must be postponed until the data are presented in full. It may be said, however, that these cases of supplementary eye-formation suggest that the formation of a single eye or of a pair under normal conditions inhibits the formation of further eyes within a certain region of the head, while in anæsthetic media this correlative inhibiting effect is not sufficient to prevent the formation of new eyes as the animal becomes more and more fully acclimated and the head grows larger.

The relation between the formation of single median eyes or paired eyes and the length of the piece in anæsthetics and in water demonstrate very clearly that even the localization of such an organ as the eye-spot is dependent, not merely on the character of the tissues from which it is formed, but upon the organization of the whole piece of which it forms a part. Moreover, the fact that, other things being equal, a longer piece is necessary for the formation of paired eyes in the anæsthetic than in water indicates that the conditions or processes in other parts of the piece are less effective in the localization of the eyes in anæsthetic media than in water.

VII. The Effect on the Whole Animal.

When whole animals of large size (15–18 mm.) are placed in the anæsthetics the first effect besides the more or less complete cessation of locomotion is usually the loss of the pharynx, which is often extruded within forty-eight hours, though it may be retained and undergo degeneration in situ. Following its extrusion or degeneration a small new pharynx is usually slowly formed in the old pharyngeal pouch, but this never attains anything like the size of the original organ. Small young animals usually do not extrude the pharynx, and, so far as I have been able to determine, it does not degenerate when retained.

The heads of whole worms in anæsthetics gradually assume the shape characteristic of heads regenerated under the same conditions. The auricles decrease in length until scarcely visible, the head becomes smaller in proportion to the body and its outlines become more rounded.

In many cases, though by no means always, the large individuals begin after a few days in the anæsthetics to degenerate in the region representing the posterior end of the first zoöid, *i. e.*, the anterior half of the postpharyngeal region, and this degeneration results in the complete separation of a posterior portion corresponding to the second zoöid and an anterior portion consisting of the first zoöid minus more or less of its posterior end. In some cases the disintegration proceeds gradually in each piece after separation until the whole is disintegrated, but very commonly it ceases after the separation of the two parts, the surfaces heal and the posterior piece develops a new head as it would do after normal fission, while the anterior piece produces a small amount of new tissue at its posterior end. This process of disintegration is not due to an infection or to any other accidental condition in the medium, as is clearly shown by two facts: first, it increases in frequency with decreasing temperature. At low temperature practically every large individual separates into two parts while in room temperatures separation often occurs in less than half. And second, such disintegration and separation almost never occurs in individuals below a certain size, either in high or low temperatures. In short we find that the posterior region of the first zoöid in large individuals is subject to degeneration and disintegration in anæsthetics, and more frequently in low than in high temperatures, while in small individuals degeneration rarely or never occurs in this region.

I can account for these facts only as follows: In the large individual the first zoöid is approaching the limit of size and its posterior end being most distant from the centers of correlation, the cephalic ganglia, is approaching a condition of what we may call physiological isolation. When we place such individuals in the anæsthetics we decrease the effective distance of transmission of the nervous processes and the posterior region of the first zoöid becomes still further isolated. This region is incapable of form-

ing a new animal in anæsthetics, as we can readily show by isolating it physically, *i. e.*, by cutting it out and placing it in an anæsthetic medium, where it almost always degenerates. Consequently its condition while still attached to the other parts is comparable to its condition when physically isolated, *i. e.*, it is then physiologically isolated to such a degree that it behaves as if physically isolated and disintegrates. When we add the effect of low temperature to that of the anæsthetic, the physiological isolation is more certainly and more completely induced, consequently disintegration of this region is more frequent in low temperatures. This process of disintegration does not occur in small individuals because they are far below the limit of size and the posterior end of the first zoöid is not physiologically isolated in anæsthetics to any such degree as in the larger individuals.

VIII. Acclimatization.

If the individuals or pieces live for a week or more in the anæsthetic they became more or less acclimated and begin to move about and react in the usual manner, though very slowly and imperfectly. In many cases they continue to live for months, especially in alcohol. In ether and chloretone I have not as yet been able to keep them alive for so long a time in concentrations corresponding in their effect on regulation to those of alcohol employed for this purpose. By beginning with mixtures of low concentration and gradually increasing the concentration they can be acclimated to concentrations which otherwise kill them within a few hours. I have not thus far attempted to determine the limit of acclimatization, since my attention has been directed chiefly to other problems. It is of interest, however, to note that young animals become more readily and more completely acclimated to mixtures of given concentration than do older ones, although the younger individuals show a higher death-rate than the older when first placed in the anæsthetic. And finally individuals which have been reduced in size by starvation do not show the same power of acclimatization as young animals of the same size, but on the contrary, their ability to become acclimated decreases as starvation and the decrease in size continue. These facts bear upon various recent attempts at

interpretation of the reduction processes as reversals of development (Lillie, Schultz and others). It is evident from my experiments that the individuals reduced by starvation, although they may be simpler morphologically, than the full-grown individuals, are physiologically not younger but older than these.

IX. Length of Life.

In an extended series of experiments I have attempted to collect data concerning the length of life in anæsthetics of constant concentration, with the result that some conclusions of interest have been reached.

As noted above, young individuals of small size are more sensitive than older, larger, and die in greater numbers during the first two or three days, but if they survive this period they very commonly live longer than the larger older animals, since they become more completely acclimated. Pieces from the posterior region of the body of large animals and corresponding to the second zoöid behave in the anæsthetics like young animals.

Animals and pieces which have been well fed up to the beginning of the experiment live longer in anæsthetics than starving individuals and pieces. It is of interest to note that the further starvation advances, the earlier do the animals die in anæsthetics of given concentration.

Pieces from different regions of the body show very different degrees of resistance to the anæsthetics. Pieces from the anterior region without the head live much longer than pieces of the same length from the middle region. Short pieces including the old head die much earlier than longer pieces and their death is not due to reduction in consequence of starvation to the limit of existence, for they die long before such reduction occurs. In other series of experiments pieces have been allowed to begin the process of regulation in water and have been placed in the anæsthetic at various stages. If we compare long and short pieces with anterior ends at the same level of the body, we find in general that, as regulation proceeds, the short pieces, which have undergone a more extensive reorganization than the long pieces, behave more and more like young animals when placed in the anæsthetic, *i. e.*, they are more sensitive at first, but if they survive they become more

readily and more completely acclimated than the longer pieces and outlive them. In short the more extensive the regulatory reorganization of the piece the younger it is physiologically, and vice versa. These facts bear upon the general problems of age and "rejuvenation."

X. Conclusion.

The above brief statement of results includes only the more important features of the experiments, but is, I think, sufficient to demonstrate the value of the method and the possibility of its application to various problems.. I hope to extend the experiments with these and other substances and with other species, in order to obtain a broader basis for comparison. I scarcely need call attention to the possibilities of the method for the analysis of morphogenetic phenomena.

The results attained by anæsthetics can be approached more or less closely and in certain respects by decreasing the supply of oxygen, by low temperature and by starvation. In this respect my experiments are in accord with the hypothesis suggested by various authorities, viz., that anæsthetics inhibit to a greater or less extent the fundamental metabolic reactions or certain of them. We shall probably find, however, on further experiment that different anæsthetics act differently in certain respects as regards the morphogenic processes.

Hull Zoölogical Laboratory.
University of Chicago,
December, 1909.

THE CHROMOSOMES OF ACHOLLA MULTISPINOSA.[1]

FERNANDUS PAYNE.

In a recent paper entitled "Some New Types of Chromosome Distribution and their Relation to Sex," I described in part the history of the chromosomes in *Acholla multispinosa*. At that time, I had but little material and was unable to say with certainty just what occurred in the second maturation division. However, I stated that all the evidence pointed to the conclusion that the ten chromosomes in the ring divided equally while the members of the hexad group in the middle remained undivided, five of them passing to one pole, and one to the other. I also stated that the number of chromosomes and their size relations in the male and female somatic cells made it almost conclusive how the members of the hexad group separated; that no other manner of distribution could give an end result of 26 chromosomes for the male and 30 for the female.

In order to clear up the doubtful points, I collected new material in larger quantities during the past summer and fortunately have been able to follow the complete history of the chromosomes in the spermatogonial, oögonial and the first and second maturation divisions. As a result, I can state that my former observations were entirely correct and that my inference in regard to the separation of the chromosomes in the second division has proven true.

The oögonial divisions (Fig. 1, *A*, *B*, *C* and *D*) show 30 chromosomes, 24 of which are approximately the same size while six are much smaller. I have examined female material from New Jersey, New York, Indiana and Illinois and they all show the same number and size relations. The spermatogonial groups (Fig. 1, *E*, *F*, *G* and *H*), on the other hand, contain 26 chromosomes, 22 medium sized, one very large and three small. Since the number and size relations of the chromosomes in the male and female groups are so different, it might be argued that I have been working with two species. In regard to this question, I

[1]Contribution from the Zoölogical Laboratory of Indiana University, No. 111.

wish to state that with this point in mind, Van Duzee has examined my material very carefully and that the larger part of it (30 specimens) consisting of both males and females was collected within a small circular area of about 200 feet in diameter.

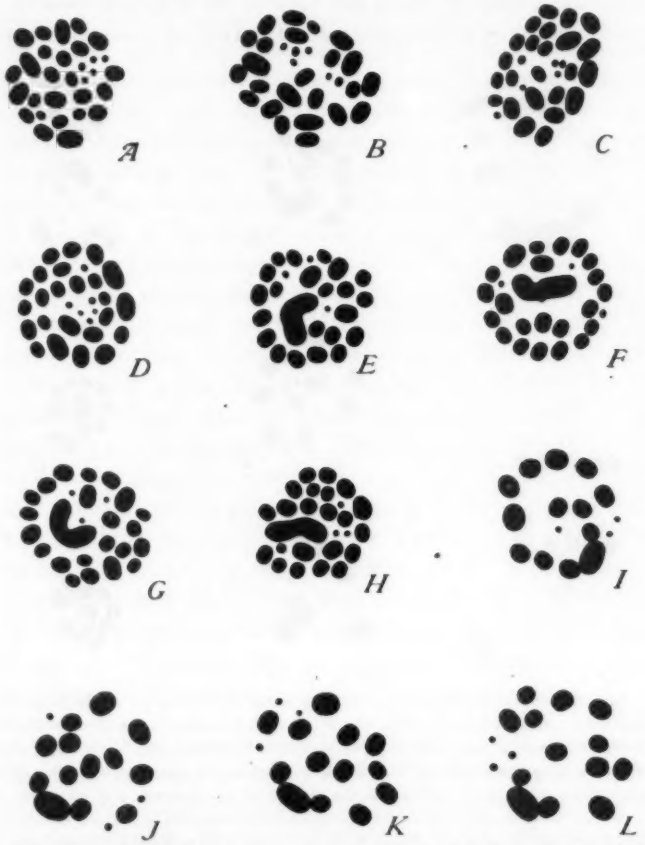

FIG. 1.[1] *Acholla multispinosa.* *A*, *B*, *C* and *D*, metaphase figures of the oögonial division, showing 30 chromosomes; *E*, *F*, *G* and *H*, spermatogonial divisions showing 26 chromosomes; *I*, *J*, *K* and *L*, metaphase figures of the first maturation division showing 16 chromosomes.

The metaphase plate of the first spermatocyte-division shows 16 chromosomes (Fig. 1, *I*, *J*, *K* and *L*). As 26 is the spermato-

[1] All figures are drawn to the same scale and magnified 1,500 diameters.

gonial number, ten of these must be bivalent and six univalent. The three small ones are again present. The large one is here as in the prophase of the first division linked end to end with two other chromosomes. All divide equally in this division. I was fortunate enough to find a side view of the anaphase showing all three small ones dividing (Fig. 2, *D*). The large one and the two

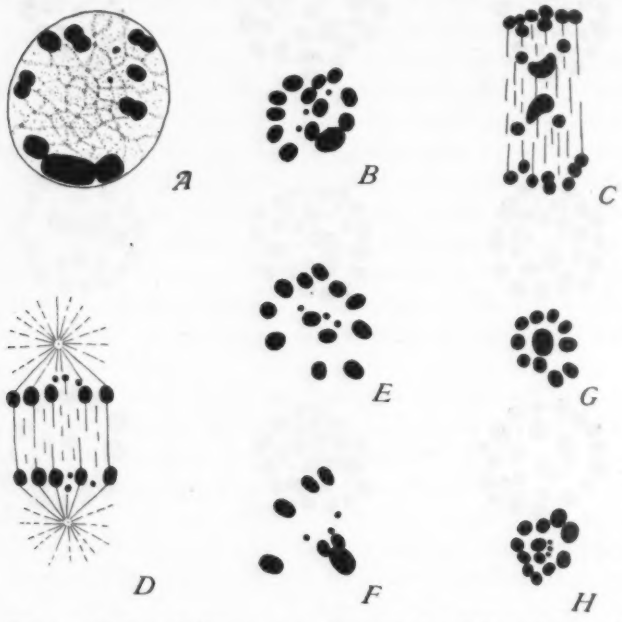

FIG. 2. *Acholla multispinosa*. *A*, prophase of the first division; the large chromosome is joined end to end with two others; *B*, anaphase, polar view, of the first division; *C*, anaphase, side view, of the first division, showing the late division of the large chromosome and the two with which it is linked; *D*, anaphase, side view of the first division, showing the division of the three small chromosomes; *E*, metaphase of the second division, polar view, showing the ten chromosomes in the ring and the five members of the hexad group which lie in one plane; *F*, a slightly oblique view of the second division metaphase showing the hexad group; *G* and *H*, anaphases, polar view, of the second division, showing the chromatin content of the two classes of spermatozoa.

with which it is joined are usually the last to divide (Fig. 2, *C*, a side view of the first division anaphase). Fig. 2, *B*, is a polar view showing 16 chromosomes. There is no definite arrange-

ment in this division, although the large one always lies on the periphery.

As a result of the equal division of all the chromosomes in the first division, the metaphase plate of the second maturation division shows 16 chromosomes. In this division, however, there is always a definite arrangement. Ten of the 16 form a more or less regular ring while the remaining six, two medium sized, three small and the extra large one, are arranged in a hexad group in the middle. Five members of the hexad group, the two medium sized and the three smaller ones, lie in one plane, while the sixth member, the large chromosome, lies either above or below the five on the other side of the equatorial plane. Fig. 2, *F*, is a view of the second division metaphase, slightly to one side, showing the arrangement of the hexad group. Fig. 2, *E*, shows the ten chromosomes in the ring and the five in the middle. The large one could not be shown without displacing it. The ten chromosomes in the ring divide equally while the members of the hexad group do not divide, but five of them, the two medium sized and the three small ones, pass to one pole and the large one to the opposite pole. The anaphases showing this unequal distribution are shown in Fig. 2, *G* and *H*. Two classes of spermatozoa are thus produced, differing in that one class contains 15 chromosomes, the other 11. Further, since the oögonial number is 30 and the spermatogonial 26, the reduced number of chromosomes in the egg must be 15 and the two classes of spermatozoa must be respectively male and female producing.

Spermatozoön 15 + egg 15 = 30 (♀)
Spermatozoön 11 + egg 15 = 26 (♂)

As stated in the previous account, the size relations of the chromosomes serve as an aid in reaching the above conclusions. If the fifteen-chromosome class of spermatozoa meets an egg with 15 chromosomes, three of which are small, the offspring should have 30 chromosomes, six of which should be small. This is what we find in the female cells. If the eleven-chromsome class of spermatozoa meets the same egg, the cells of the embryo should have 26 chromosomes, three of which should be small and one extra large. This condition is fulfilled in the male cells.

I have not been able to follow the history of the differential chromosomes during the growth period. A plasmosome is present. Sometimes it stains perfectly black but again it may stain much as the cytoplasm does. If the latter, a number of chromatin bodies can be seen embedded in it. As I have followed the history of the plasmosome and its relation to the differential chromosomes in *Prionidus* it seems very probable that here, too, in *Acholla* the differential chromosomes are embedded in the plasmosome during the growth period.

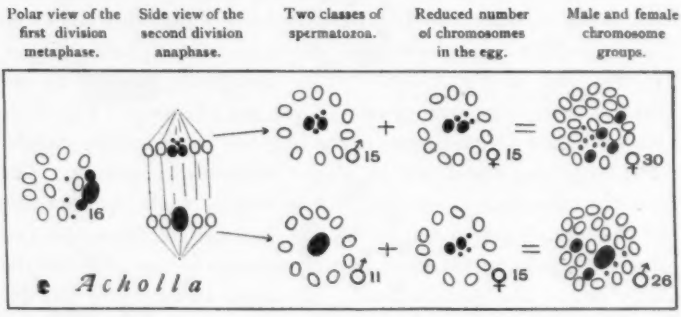

FIG. 3.

Fig. 3 gives a diagrammatic representation of what occurs in maturation and fertilization.

Only a few years ago maturation meant a reduction to one half; the number of oögonial and spermatogonial chromosomes were thought to be the same in each species and this number an even one. Even as late as 1900, Wilson in "The Cell in Development and Inheritance" writes as follows: "Van Beneden's epoch-making discovery that the nuclei of the conjugating germ-cells contain each one half the number of chromosomes characteristic of the body-cells has now been extended to so many plants and animals that it may probably be regarded as a universal law of development." Those who believe that the odd chromosome is merely a delusion in the minds of a few investigators still cling to the universality of Van Beneden's law. However, the law is no longer of universal application. Not only the odd chromosome but a number of other irregularities have been recently described,

the present case of *Acholla* giving the greatest variation in number. In fact so many variations have been found that we may justly ask what is to be the limit of these irregularities and where will they lead us?

The case of *Acholla* is interesting for two principal reasons. First, it gives us the greatest variation in number so far discovered, there being a difference of four between the male and female groups. Secondly, if we examine the size relations carefully it will be noticed that the large chromosome which goes to the male-producing pole seems to contain a larger amount of chromatin than the five chromosomes of the hexad group which go to the female-producing pole. A number of measurements show this to be the case. In all the irregularities hitherto described, the female cells contain the larger number of chromosomes and also the greater quantity of chromatin. The present case is not an exception to the number but seems to be in regard to the quantitative relations.

Several recent theories of sex determination have been based upon the quantitative relation of the chromatin. The evidence in *Acholla* forms one of the stumbling blocks in the way of some of these interpretations. To be sure, if we should ignore the large chromosome, the homologue of the small idiochromosome, or what Wilson terms the *Y* element, many difficulties would disappear. Most certainly though, as Morgan says, there is no reason for disregarding it except that its presence, in an active condition, does not fit in with our hypothesis. In his recent paper on "Sex Determination in Phylloxerans and Aphids," Morgan while realizing the difficulties in its way, further develops the quantitative hypothesis and holds to it as a rough approximation to a solution. In regard to the case of *Acholla* he suggests that possibly the five chromosomes of the hexad group which go to the female-producing pole are more active than the large chromosome which goes to the male-producing pole. The suggestion may be true, but is the question of the activity of the chromosomes the same as that of the quantitative relations?

THE POLE DISC OF CHRYSOMELID EGGS.

H. L. WIEMAN,

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Hegner (09*b*)[1] has described a disc-shaped mass of darkly staining granules at the posterior end of freshly laid eggs of two genera of beetles, *Calligrapha and Leptinotarsa*. In earlier publications (Hegner, 08, 09*a*)[2] these granules are spoken of as "germ cell determinants" in the sense that they fix the character of the sex cells. The use of the word determinant is open to criticism inasmuch as the term implies the attribute of certain potentialities that these granules have not been shown to possess. In the complete account of the early history of the germ cells (Hegner, 09*b*)[1] the word *determinant* does not appear, and the conclusion regarding their significance is summed up in the statement, that the "pole-disc" is "intimately associated with the development of the pole cells" (p. 288).

In view of the experiments of Lyon, Lillie, Morgan and others, which center about the question of the rôle of preformed materials in the egg as *versus* a predetermined method of action as the essential factor in embryonic development, it is important to know something of the nature and origin of the granules of the pole disc. The data derived from the experiments of these investigators tend to indicate that early developmental phenomena can take place even though the original configuration of the ground substance be radically changed. The formative processes do not depend on the materials displaced by centrifuging, but are bound up in the organization of the ground substance of the egg protoplasm.

Hegner did not determine the origin of the pole disc, but is inclined to believe that it is composed of particles of chromatin derived from the nucleus. No expulsion of material from the nucleus was actually observed; the conclusion being based

[1] *Journal of Morphology*, XX., 2, 1909.

[2] BIOLOGICAL BULLETIN, XVI., 1, 1908; *Journ. Exp. Zoöl.*, VI., 4, 1909.

mainly on staining reactions and the results of Blochmann (86),[1] Stuhlmann (86),[2] and others who have described in various species of hymenoptera a budding of the nucleus of the ovocyte resulting in the formation of many small "nuclei" (Nebenkerne) each containing small dark staining granules. The work of Sylvestri (08)[3] is also cited as supporting a nuclear origin of the pole disc.

In connection with some work embodied in a paper now in press, I have had occasion to study the process of nutrition in the egg of *Leptinotarsa signaticollis*, a chrysomelid beetle closely related to *L. decemlineata*, one of the species studied by Hegner. In this form the nutritive material consists of a granular mass secreted by the nurse cells of the ovariole. This material enters the egg through the egg-string which is a protoplasmic process of the egg terminating in the groove-like spaces between the nurse cells. In safranin-lichtgrün preparations these granules stain as intensely with the basic dye as the granules of the pole disc.

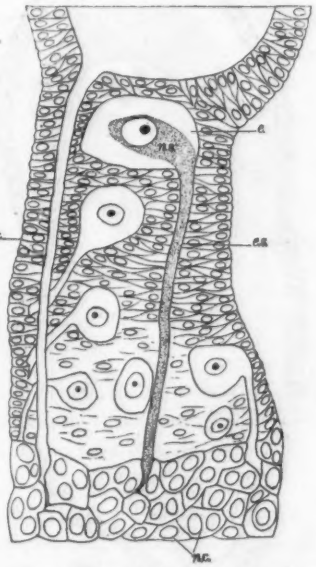

FIG. 1. Longitudinal section of an ovariole showing ovocytes at the beginning of the growth period. The nutritive stream (*n.s.*) is represented in only one of the eggs (*e*). *e.s.*, egg string; *n.c.*, nurse cells; *o.s.*, ovariole stalk.

Fig. 1 is a semi-diagrammatic drawing representing a longitudinal section through an ovary containing developing eggs. The eggs which in an earlier stage are massed together in the proximal region of the terminal chamber of the ovariole, gradually become arranged in a linear series in the ovariole stalk, the cells of which form the egg-follicles. The egg-strings elongate as the eggs move down into the follicles,

[1] *Festschr. Nat. Med. Vereins. Heidelberg.*

[2] *Ber. d. Nat. Ges. Freiberg*, I.

[3] *Bolle. Lab. zool. gen. e agr. della R. Scuola Superiore d' Agricoltura di Portici*, III., 1908.

and pass back from the proximal end of the egg (which represents the anterior end of the future embryo) to the nurse cells.

When the egg is at the stage of development shown at *c*, the food material passes in a broad stream toward the nucleus beyond which it extends for a short distance. A little later (Fig. 2) the form of the nutritive stream changes so that now it encloses a central area of newly formed yolk. The nucleus has shifted its position to the distal (posterior) end of the egg, where it appears as a rounded body composed of an acid staining ground substance in which a number of basic staining nucleoli are embedded.

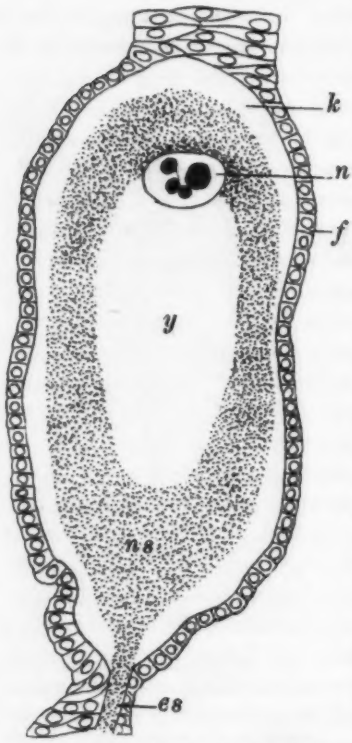

FIG. 2. Longitudinal section of a half-mature ovocyte. *e.s.*, egg string; *f.*, egg follicle, formed by the cells of the ovariole stalk; *k.*, Keimhaut; *n.*, nucleus; *n.s.*, nutritive stream.

When a portion of the cytoplasm containing the food stream is examined at a high magnification, granules of different sizes are seen distributed on an irregular reticulum (Fig. 3). The granules at their point of entrance into the egg as well as those found in the cytoplasm of the nurse cells are of a uniformly small size; while inside of the egg they are of various sizes, due either to coalescence of the small granules or to an actual growth of individual granules. These granules now pass through an interesting cycle of stages. When they have attained their full size they stand out from the reticulum and project into the interreticular spaces (Fig. 4, *a*). They now divide (Fig. 4, *b*). This division is very exact and divides each spherule into two bean-shaped halves.

A series of divisions now ensues until a number of bodies, like chromosomes in appearance, is produced in a single space (Fig. 4, *c*). After about four or five divisions have taken place, they begin to lose their affinity for the basic stain and also their regularity of outline (Fig. 4, *d*), and in the next stage (Fig. 4, *e*) they are seen to have fragmented into numerous irregular bodies that stain with the acid dye. Still later (Fig. 4, *f*) the space is practically filled with a finely granular acid staining yolk mass.

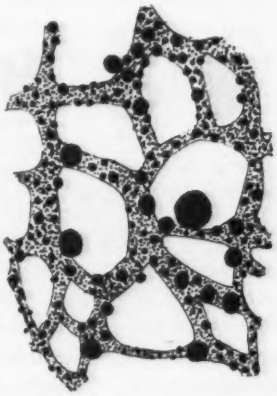

FIG. 3. Section of the cytoplasm of the growing ovocyte in the region of the nutritive stream. × 2,000.

The process of yolk formation, therefore, consists in the transformation of the basic staining granules of the food stream into an acid staining yolk which fills the meshes of the cytoplasmic reticulum of the mature egg. In this transformation the cytoplasm as well as the nucleus is undoubtedly involved. The yolk is first laid down in the center of the egg, but as the granules spread outward as well as inward from the nutritive stream it is not long before yolk is found on either side of the food stream.

The pole disc does not appear until the egg is nearly mature, and a study of successive stages shows that it is composed of granules of the food stream that have accumulated at the posterior end of the egg (Fig. 5). These granules are much larger than those of the Keimhaut (the peripheral layer of cytoplasm surrounding the yolk in the mature egg) and stain intensely with the basic stain. This staining reaction might suggest that the granules are of nuclear origin; but while the nucleus at this time is far from inactive, with an interchange of materials between it and the cytoplasm probably taking place, I have never been able to observe an actual emigration of granules from the nucleus into the cytoplasm.

Union of the germ nuclei in fertilization and the early cleav-

ages take place approximately in the center of the egg. As cleavage proceeds there occurs a separation of the nuclei into two groups, one of which moves outward into the peripheral cytoplasm to form the nuclei of the "Keimhautblastem," while the

FIG. 4. Six stages in the division of a spherule of the nutritive stream in the process of yolk formation. × 1,200.

other (the vitellophags) remains scattered through the yolk. According to Hegner a definite number of nuclei reaching the posterior part of the egg do not remain in the peripheral cytoplasm, but collect about them a number of granules from the pole disc and continue their migration until they are entirely separated from the blastoderm. These nuclei with their accumulations of cytoplasm constitute the primordial germ cells.

Fig. 6 represents a section taken through the region of the pole disc shortly after the pole cells have reached the periphery. It will be noticed that the granules of the pole disc are much finer than in the earlier stage shown in Fig. 5. The granules in the

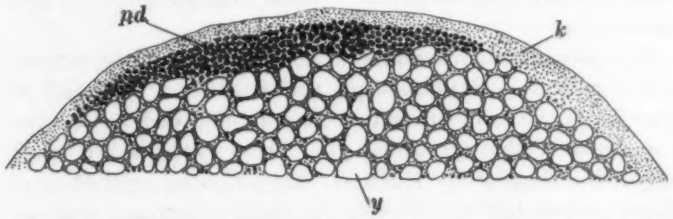

FIG. 5. Longitudinal section of the posterior end of a mature ovum. × 1,000 *p.d.*, pole disc; *k.*, Keimhaut; *y.*, yolk.

cells are distributed on a reticulum and stain intensely with the basic dye, but no more so than the granules that are found in the cytoplasm of the blastema cells. In one case the granules are derived from the pole disc, while in the other they come direct from the food stream. The original source in both cases is the material secreted by the nurse cells.

Does the fact that the granules of the pole disc are derived from the food stream in any way modify our conception of their significance? Were they derived from the chromatin of the nucleus, the fact might be regarded as supporting their hypothetical rôle of germ cell determinants, inasmuch as it would be in accord with the view that the chromatin plays a primary part in directing and perhaps determining the activity of the cell. On

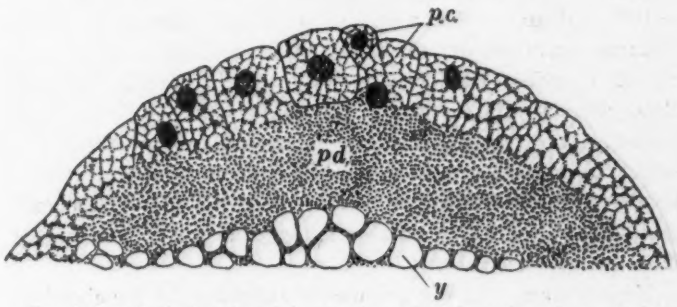

FIG. 6. Longitudinal section of the posterior end of the early embryo. × 600. *p.c.*, pole cells; *p.d.*, pole disc; *y.*, yolk.

the other hand, if they represent so much food material, their significance as germ cell determinants appears in a different light.

The granules of the food stream are derived from the cytoplasmic granules of the nurse cells, which are germ cells that have lost the function of reproduction and that have taken up secondarily the function of secreting material for the growth and development of the ova. In early stages the nurse cells do not differ in any regard from their sister cells destined to become ovocytes. In my paper referred to above I have described what appear to be some of the factors in the differentiation of the primordial germ cells into nurse cells and sex cells, and shall not enter into the matter here.

At the time of this differentiation the nurse cells pass through a period of amitotic divisions, at which time the usual staining reactions of nucleus and cytoplasm become reversed, *i. e.*, the nucleus stains with the acid dye, while the cytoplasm, including the granules which later form the nutritive stream, stains with the basic dye.

The granules may therefore be of the nature of chromatin, and actually represent the chromatin of the nurse cells; for the chromosomes never appear in these cells after they have divided amitotically. But even though this be the case a nuclear origin for these granules loses any special germinal significance when we remember that the latter develop into yolk as well as pole disc, and are therefore just as much "yolk determinants" as "germ cell determinants."

It would appear that a fact of far greater importance in the determining of the germ cells lies in the migration of the pole cells to a position outside of the yolk. This migration isolates these cells and places them in an environment that is entirely unique. It is rather difficult to understand how the absorption of pole disc granules could be the cause of this. The fact that the pole disc occupies a position between the pole cells and the yolk gives a considerable foundation for regarding it as a source of nutrition for these cells.

Hegner states that the granules of the pole disc are absorbed by the pole cells in passing through; after which no further importance is attached to the pole disc. However the granules are not all taken up by the cells in their migration and the greater part of them remains behind after the cells have passed through (Fig. 6).

If then the pole disc represents a part of the nutritive stream of the ovum that has not been transformed into ordinary yolk, but instead has been reserved to supply the pole cells, the conclusion presents itself that the latter as a result of this special kind of nutrition undergo a peculiar method of metabolism which differentiates them from the somatic cells. When these cells are ready to immigrate into the embryo through the pole-cell canal the differentiating factor has already acted and the germ cells are readily distinguished by certain morphological peculiarities from the somatic cells.

It would be highly interesting to know whether or not the germ cells will develop in the absence of the pole disc. The experiments devised by Hegner to test this point have been negative in results, although they do show that the egg may have its contents profoundly disturbed without preventing the production

of a normal embryo. When the granules of the pole disc are displaced by the centrifuge, they are said to move *en masse*, which indicates that they form a structural feature of considerable rigidity.

While more decisive experiments are needed to clear up this point, the evidence at the present time from the morphological side shows that the granules of the pole disc consist of particles derived from the food stream of the ovum that form an accumulation in the protoplasm in its posterior part.

FORTY-NINE GENERATIONS IN THE DARK.[1]

FERNANDUS PAYNE.

Since the time Lamarck put forward the well-known theory of the transmission of acquired characters, this subject has been discussed pro and con by many writers; some believing that it is one of the guiding principles in the evolution of species; others that the transmission of an acquired character is an utter impossibility. I shall not attempt a review of the literature, as every book on heredity and evolution has its chapter devoted to that. In fact, it seems to me that there has been too much discussion and not enough experimental work. I believe it can justly be said that there has not been a single decisive experiment which proves or disproves the theory. They are all open to criticism in one way or another. The main argument which the opponents of the theory advance is that there has been no proof brought forward, and further there is no conceivable way in which an acquired character could be transmitted. On the other hand, the supporters believe that this is one of the easiest ways of explaining evolution and as it helps us out of many a difficulty it must be true.

Much interesting data has been collected, but none of it is conclusive. What we need at the present time is more experimental work to test the validity of the theory. With this attitude toward the subject, I started an experiment October 21, 1907, while at Columbia University to test the effect of darkness upon the common fruit fly, *Drosophila ampelophora*, and, if any effect was noticeable, to test its transmissibility. This seemed to be a suitable experiment as the present condition of cave animals is easiest explained by the assumption of the transmission of acquired characters.

The paper is not a finished report, but it may be of interest to scientific men to learn that such an experiment is in progress, and that this fly has been bred in the dark for forty-nine generations. Most certainly the length of time is rather short, but the

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 112.

number of generations is large when compared with longer lived animals. Forty-nine generations of mankind would cover about fifteen centuries of time.

The first changes noticeable in cave animals are the loss of color and the degeneration of the eyes. So far, in the case of the flies, no visible change in color has manifested itself. Sections of the eyes showed all parts perfectly normal. Although I have not succeeded in getting an accurate method of measuring it, there is a noticeable difference in their reactions to light. These flies are positively phototactic and if set free nearly always fly toward the light. Those bred in the dark are still positive, but they do not react so quickly nor do so many of them react. At the end of the tenth generation, this difference was noticeable. So noticeable, that when two vials, one containing flies bred in the light and the other in the dark, were shown to several people at the laboratory without them knowing what they were, they immediately remarked that one lot went toward the light much more quickly than the other. This tenth generation, after being bred in the light for one generation, still showed a difference but not quite so marked. Whether or not the flies of the forty-ninth generation are more sluggish in their reactions to light than those of the tenth generation is impossible for me to say as my method of testing is not sufficiently accurate. I expect to continue the experiment and hope later to devise a method by which I can test the reactions of each fly individually and thus see whether the effect of the darkness is a cumulative one.

Much discussion has centered around the question of the origin of cave faunas and several suggestions have been made in regard to its solution, but it seems pretty generally concluded that animals which now inhabit caves have entered them because they were originally dark-seeking forms. The present experiment shows that a positively phototactic animal might establish itself in a cave if it were accidentally enclosed in such a place and if a suitable food supply were present.

Since no changes have been produced in the color of the body or in the structure of the eyes by breeding the fruit fly in the dark for forty-nine generations, the question arises as to whether the length of time which these flies have been bred in the dark

is too short or whether they have reached a fixed condition in so far as variations toward adaptations for a cave life are concerned. We might extend it a little further and ask whether all animals would lose their color or whether their eyes would degenerate in a cave environment? In other words, is the environment alone responsible for the present condition of cave animals or is there an internal orthogenetic factor at work to which the environment serves as a stimulus? Eigenmann in his discussion of the origin of cave animals has stated that forms which now inhabit caves were predestined to become cave animals long before they ever entered a cave. That is, they had varied in a certain fixed direction, and these variations had tended to fit them for a cave existence. If such is the case, would the present cave animals have become blind and colorless if they had not entered caves?

DO BLOW FLY LARVÆ RESPOND TO GRAVITY?

S. O. MAST.

Referring to the fact that blow-fly larvæ crawl under objects in water just as they do outside, Loeb says (1905, p. 68):[1] "In these experiments I was struck by the fact that the animals, when placed under the surface of the water, do not swim upward and so avoid death, but swim downward. I cannot explain this fact. Under other conditions positive geotropism cannot be demonstrated in these animals." Relying on this statement of Loeb, I attempted to ascertain the cause of the expressed difference in response to gravity of fly-larvæ in air and in water and the method of orientation in swimming downward. The result of this investigation follows.

In making observations on the reactions of blow-fly larvæ in air, twenty-five active specimens varying in length from 5 to 17 mm. were put into a glass jar with vertical walls so situated that the light intensity in the jar was very low and approximately uniform throughout, thus eliminating its influence on the direction of movement. In the course of several minutes it was found that nearly all the larger specimens were crawling almost straight upward on the sides of the jar, apparently responding negatively to gravitation. The smaller larvæ however crawled about in all directions, horizontally and downward as well as upward. What is the cause of this difference in the direction of locomotion?

Careful observation soon showed that while most of the larger larvæ observed at any given time were directed upward, they frequently turned and started in other directions, but that as soon as they got into a position approaching the horizontal they either fell to the bottom of the jar or the posterior end swung downward somewhat every time it was raised in the process of looping, and that this resulted in orientation with the anterior end directed upward.

Thus it is clear that there is no evidence of a reaction to gravitation in these forms under the conditions of these observations, *i. e.*, in air. Do they become positive to gravity in water and swim downward as Loeb says, and if so, how do they orient?

Larvæ approximately 6 mm. long were taken from carrion

[1] Studies in General Physiology, Vol. I., 423 pp., Chicago.

and put into a glass jar containing water 20 cm. deep. They all soon reached the bottom, but there was not the slightest evidence of swimming downward. They did not orient and the longitudinal axis was nearly horizontal in many of them. In fact there was but little bending and twisting in the larvæ. Most of them remained perfectly quiet as they sank to the bottom and none of them deflected more than 2 cm. from the vertical. The same was true for larvæ approximately 1 cm. and 1.6 cm. long taken from the carrion and put directly into the water on the second and fifth days following. But in case of other specimens which were kept in a dish without food for several hours before they were put into the water many did not sink. This was due to the formation of small bubbles of gas near the anterior end.

Some of the larvæ which had thus been without food were killed in boiling water, others in alcohol. Of the dead specimens some sank, others floated. The dead ones were now mixed with living ones, and all put into a small glass jar containing water 80 cm. deep. After the larvæ which were heavier than water had sunk to the bottom, the jar with the upper end closed was suddenly inverted. Those which had been at the bottom sank again, while those which had been at the top rose. In both lots there were some living and some dead specimens, but it was only with great difficulty that one could distinguish them. In nearly all specimens the axis was approximately horizontal. There was no evidence whatever of a swimming movement in any of them. If there is no gas in them they sink to the bottom where they crawl about much as they do in air. If they contain bubbles of gas they float at the surface, where in one instance they were observed to remain alive more than twenty-four hours.

It must therefore be concluded that blow-fly larvæ do not react to gravity, either in water or out of it. In air they may be found to orient and crawl nearly straight upward on objects, but this is not due to a response to gravity on the part of the organisms. In water they sink to the bottom or float at the top, depending upon the amount of gas they contain, but there is no evidence whatever indicating that they can swim.

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PECULIAR HABITAT OF A PYCNOGONID (ENDEIS SPINOSUS) NEW TO NORTH AMERICA, WITH OBSERVATIONS ON THE HEART AND CIRCULATION.[1]

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During the summers of 1904, 1905 and 1906, sargasso, or gulf-weed, at various times drifted into Vineyard Sound in considerable abundance. This is the case some years, while others it may seldom be seen or may be wholly absent during the entire season. Gulf-weed is borne northward each season in large quantities by the Gulf Stream, which here lies nearly a hundred miles off the coast, and its appearance in these in-shore waters would seem to depend in large part upon the occurrence and prevalence of southerly and easterly winds, which divert a certain amount of it shoreward from its course further out. In 1904, and presumably also in the two succeeding seasons, although my records do not state specifically as to this, the weed was covered with a delicate but very abundant growth of the hydroid *Obelia dichotoma* (Linn.).[2] Living among the colonies of this hydroid, and clinging tenaciously to its stems and branches, occurred a slender pycnogonid, a species of *Endeis*, both adult males (many of them carrying eggs) and adult females, as well as young in various stages of growth, being found in great numbers.

The largest of these pycnogonids were of a considerable size, being approximately 15 mm. across from tip to tip of the extended legs; but all were nearly white, or of a light straw color, in general appearance, which rendered them far from conspicuous among the similarly colored hydroid stems. Examined individually, with more care, it was found that the intestine and its diverticula in the legs usually appeared greenish, probably due to its contents, while there was a pinkish tinge at each of the articulations of the different joints (articles) of the legs. When

[1] The observations reported in this paper were made in the laboratory of the United States Bureau of Fisheries at Woods Hole, Mass.

[2] The identification of this species was kindly confirmed for me by Professor C. W. Hargitt.

the intestine was empty, the legs often appeared white at the joints (articulations) and transparent between. The distal half of the proboscis is usually the most conspicuous part of the animal, being a tinge or shade of yellow, in some cases so dark as to be almost buff. Eyes dark reddish brown.

While this pycnogonid was found plainly to belong to the genus *Endeis* (*Phoxichilus*[1] of authors), its peculiar occurrence among the gulf-weed, and the fact that the genus had never been reported from American waters, led to the suspicion that it would prove to be specifically distinct from any described form. Comparison, however, with descriptions of *Endeis spinosus* (Montagu) of the European coast showed so little discrepancy that I have delayed coming to a definite conclusion until I should be able to compare it directly with specimens from that locality. This I have now been enabled to do through the kindness of Prof. G. O. Sars, of Christiania, Norway, and Mr. T. V. Hodgson, of Plymouth, England, who have generously supplied me with

[1] The name for this genus has been commonly known and accepted as *Phoxichilus* since 1837 at least. It now appears that the first form described as a *Phoxichilus* in reality belongs to Wilson's genus *Pseudopallene*. According to the ironclad "law of priority" therefore, the name *Phoxichilus* must now be used in place of *Pseudopallene* for that genus, and hereafter all but specialists on Pycnogonida will find what is to them an almost hopeless confusion of names if they ever have occasion to look up the literature on such a subject, say, as the circulation of the blood in this group, mentioned later in this paper. Stebbing (1902, p. 187) proposed the name *Chilophoxus* to take the place of *Phoxichilus* for those animals which have been universally known by the latter name. But the evil ball once started rolling could not be stopped here, for Norman (1908, p. 231) has pointed out that undoubtedly the first species described by Philippi (1843) under his genus *Endeis* "is congeneric at least, if not identical, with *P*[*halangium*] *spinosum* Montagu," which has so long been known as *Phoxichilus spinosus*, and is the subject of the present note. This being the case (and there would appear to be no doubt of the facts) *Endeis* of course takes precedence over *Phoxichilus*, and the name must therefore stand as *Endeis spinosus* (Montagu).

When I began systematic work on the Pycnogonida, more than ten years ago, I believed implicitly in the advantages to be gained by following strictly the law of priority; after considerable experience in observing its effects, I am inclined to agree with Thompson (1909, p. 537, footnote) when he remarks with regard to the instance just discussed: "In my opinion this is a case where strict adherence to priority would serve no good end, but would only lead to great and lasting confusion." However, after much mental contention, I have decided for the present to be law-abiding, and to live in the hope that before long an agreement may be reached among zoölogists permitting the use of common sense in place of forcing blind adherence to a law which, however good its intention, has seemed only to increase the instability and confusion in zoölogical nomenclature.

specimens of undoubted *Endeis spinosus* from the Norwegian coast and from Plymouth, respectively. Furthermore, I have had one specimen, labelled "*Phoxichilus vulgaris*," which I feel confident came from Naples, although no locality was given on the label. My reasons for believing that this specimen came from there are twofold: First, because it was with other Mediterranean pycnogonids, and second, because the name *vulgaris*, which European writers unanimously consider as a synonym of *spinosus*, has been used for this species only in that locality. Finally, in addition to those from Vineyard Sound I have from the American side of the Atlantic a number of specimens of *Endeis* from the Tortugas, Florida. One of these I collected myself; the others were kindly sent to me by Dr. A. G. Mayer, director of the Marine Laboratory of the Carnegie Institution.

After having compared carefully the specimens from these five widely separated localities, viz., Naples, Plymouth, Norway, Vineyard Sound and the Tortugas, I am forced to the conclusion that in spite of their range and their difference in habitat, they in reality must all be considered as belonging to a single species, *Endeis spinosus* (Montagu). The Vineyard Sound specimens, it is true, appear in general a little stouter than those from Norway, due to the legs being proportionately a trifle shorter; this difference, however, is not great and is inconstant. The following table presents a number of proportions, based usually on the average of measurements of all the legs of from one to three or four specimens from each locality. The numbers of specimens measured were so small and the results are in general so irregular that not much importance can be attached to them. It will be noticed that in the proportion of the length of the femur to that of the coxal region, there is a gradual relative shortening of the femur as one goes from Naples up the European coast and down the American side to the Tortugas; the same is true in a general way for the relation of the trunk to the proboscis; while the relation of the trunk to total leg length is more irregular. This gradation in two of the cases may or may not have any real significance.[1]

[1] Owing to the comparatively small amount of differentiation in the four pairs of walking legs of the pycnogonids and to the fact that they present characters

Proportionate Measurements of Endeis spinosus from Different Localities.

Localities.	Ratios.		
	Coxa to Femur.	Leg to Trunk.	Proboscis to Trunk.
Naples	1 : 1.80	1 : 3.55	1 : 2.35
England	1 : 1.70	1 : 4.14	1 : 2.01
Norway	1 : 1.68	1 : 3.85	1 : 2.14
Vineyard Sound, ad.	1 : 1.66	1 : 4.15	1 : 1.83
" " juv.	*1 : 1.32*	*1 : 3.26*	*1 : 2.03*
Tortugas, ad.	1 : 1.43	1 : 3.74	1 : 1.92
" juv.	*1 : 1.29*	*1 : 3.53*	*1 : 1.89*

Of even more interest than the mere fact of the occurrence of *Endeis spinosus* on the American coast is its peculiar pelagic habitat on this side of the Atlantic. So far as I am aware, a pelagic habit among the Pycnogonida has not heretofore been reported, either for this species, which in Europe has been recorded as dredged from shallow depths, or any other. In a previous paper (Cole, 1901, p. 197) I have called attention to the fact that *Pallene brevirostris*, a very slender pycnogonid which occurs at Woods Hole, swims actively if by chance it becomes free in the water; but ordinarily it lives among the hydroids and algæ growing attached in shallow water. Curiously enough the specimens of *Endeis* from the Tortugas sent me by Dr. Mayer are all recorded as having been obtained from the "surface" or in the "tow." And in addition to these he has also sent me several specimens of *Pallene* and *Nymphon* (species as yet undetermined) which were obtained in the same manner. While enjoying the privileges of the Carnegie Laboratory at

(linear dimensions) which can be measured with ease and accuracy, it would seem that these animals present exceptionally favorable material for a statistical study of differentiation and correlation of these appendages. What observations and measurements I have made would seem to indicate, for example, that the ratio of the length of the legs to the body, and the correlation between the legs themselves, varies with the age of the animal, and that there is a positive correlation between the age of the individual and the position of the legs from behind forward. In other words, the posterior pairs of legs present more juvenile characters than the anterior ones. Such a study could be made profitably only on a species which could be obtained in abundance at all stages of growth. The species under consideration would be excellent for such a study, and *Anoplodactylus lentus* is another that may be obtained abundantly at Woods Hole. Whatever else of general interest might be learned, the results would be of the greatest value to the systematist in this group, who has usually to deal with a small number of specimens taken at scattered localities.

the Tortugas in 1906, I myself obtained a live specimen of *Endeis spinosus*, the animal appearing in a finger bowl which I had just filled with sea water by means of a hand pump used for that purpose, thus showing that the pycnogonid must have been swimming freely in the water when it was sucked in by the pump. Whether these pycnogonids which were taken at the surface had merely become accidentally detached from their regular abodes, or whether certain species naturally swim freely in the water at certain times, I am not prepared to say.

In the present connection the reaction of certain pycnogonids to light is of considerable interest. I have shown (Cole, 1901) that *Anoplodactylus lentus* and *Pallene brevirostris*, both of which can sustain themselves in the water by swimming,[1] move uniformly toward a source of light of moderate intensity (diffuse daylight). I was, furthermore, able to prove the same thing for *Endeis*, at least for the one specimen mentioned above as secured by myself at the Tortugas, the reaction being in all respects similar to that previously described for the other genera. The biological significance of this reaction in the case of *Endeis*, living among the hydroids on the gulf-weed, is at once apparent, for any individual which by chance became detached from the hydroid would, in response to the light coming from above, swim upward instead of going down into the deeper water, and would in this way stand a much better chance of again finding suitable attachment. From the fact that the young of all stages appeared to be clinging as tenaciously to the hydroids as the adults, I am inclined to believe that they probably do not have a definite free swimming stage, but swim only when circumstances make it necessary.

The newly hatched larvæ possess strongly developed chelifores and stout chelæ by which they attach themselves to any available object. Normally they remain clinging to the egg cluster on the legs of the male for some time after hatching. The usefulness of these effective grasping organs is readily apparent, since without them the larvæ would almost inevitably become detached and lost from their floating abode. I have elsewhere (Cole, 1904, p. 316) pointed out the usefulness of the chelifores

[1] The " swimming " consists simply of a vigorous *kicking*.

in preventing pycnogonid larvæ from being swept off the hydroids by a swift tide. After metamorphosis the claws of the walking legs are used for attachment, while the chelifores then probably serve as feeding organs, as has been observed to be the case in *Anoplodactylus* (Cole, 1906) and *Phoxichilidium* (Loman, 1907).

It seems worth while to call the attention of any who might care to investigate the embryology or anatomy of this group to the advantages of this species should it continue to appear in Vineyard Sound, where it is so readily accessible to the marine laboratories at Woods Hole. During the seasons it was observed all stages for a study of the embryology and later development were present in great abundance, while owing to its transparency, it is very favorable for observing the internal anatomy, as well as some of the physiological functions of the living animal. The peristaltic movements of the intestinal ceca in the legs are easily discernible, and our knowledge of the circulation of the blood in the pycnogonids has been derived largely from this species. The passage of food through the intestine and the extrusion of feces has been well described by Loman (1907) in the case of *Phoxichilidium femoratum*.[1] The question of the circulation has been touched upon by a number of observers, being most thoroughly considered by Dohrn (1881), but as this author makes no mention of earlier writers, it may be well to give here a brief résumé of the literature on the subject, together with a few additional observations.

Johnston (1837, pp. 374 and 379) appears to have been the first to mention a circulation of the fluids in the pycnogonid, his observations being made on the living "*Orithyia coccinea*" (= *Phoxichilidium femoratum*). Johnston, however, apparently mistook the branching intestine for blood vessels, for he says (p. 374): "The circulating system is probably reduced to a single vessel which occupies the centre of the thoracic segments, and sends a branch to each member or limb, in which the blood has an irregular movement, but cannot be said properly to circulate." On p. 379 he adds that "there is no heart." Milne-Edwards, in his "Histoire naturelle des Crustacés" (1840, p. 531), dismisses

[1] *Phoxichilidium femoratum* is well known on our coast to the northward of Cape Cod.

the matter with the statement that there exists "une circulation vague." According to de Quatrefages (1845, pp. 75, 76) no heart or vascular system exists, but the blood is agitated back and forth in part by the movements of the legs and also by the muscular movements of the intestine, which he believed to lie freely in the internal space of the legs. Van Beneden (1846, pp. 72, 73), observing a living *Nymphon*, was the first to make out a regular circulation. He states that the blood may be seen to flow down one side of a leg and back the other, then into the next following leg, and so on to the last pair, after which he could not tell what course it takes. On account of the opacity of the intestine he could not determine whether there was a heart or dorsal vessel present, but he observed a contractile membrane at the bases of the legs. (It is probable that what he saw were the valves of the heart where that organ extended beyond the outline of the intestine.)

Zenker (1852, pp. 382, 383), also studying *Nymphon*, was apparently the first really to see the heart. He describes it as a thin-walled sac with ramifying muscle fibers, the contour being most clearly discernible in the region of the last pair of legs. Three years later Krohn (1855) published a much fuller description of the circulation, together with a figure of the heart of *Endeis spinosus*. Hoek (1881*a*, 1881*b*) described the structure of the heart in *Colossendeis* and certain other forms. According to him there are ordinarily three pairs of lateral ostia (the posterior pair being very close together) except in *Pallene brevirostris*, which has but two pairs. In the same year Dohrn (1881) published the best description of the structure of the heart and of the circulation that has yet been given, his description being based principally again on *Endeis*. He found here two pairs of lateral ostia, with commonly, but not always, an unpaired aperture at the posterior end. There are no blood vessels aside from the heart, but the blood driven out from the anterior end of the heart is forced into the proboscis and runs back along the ventral side of the body, and from here it flows out into the legs. In general the direction of flow is centrifugal on the ventral side and centripetal on the dorsal side (except in the proboscis, where it is just the reverse). A thin membrane, which supports the

intestine, keeps the two streams apart (cf. Fig. 2, *sept.*). Dohrn's description of the heart agrees very closely with that of Hoek, it being an elongated sac with thin muscular walls, which, how-

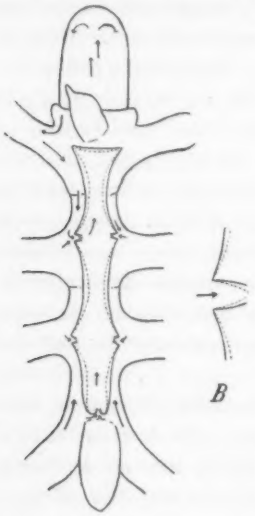

FIG. 1. *A*, apparent change in the shape of the heart with contraction and expansion, as seen when viewed from above in a living specimen of *Endeis spinosus*. Solid line, diastole; broken line, systole. The arrows show the course of the blood where its streaming could be observed; the opacity of the intestine and other organs prevented its being seen in other parts. *B*, a single lateral ostium with valve.

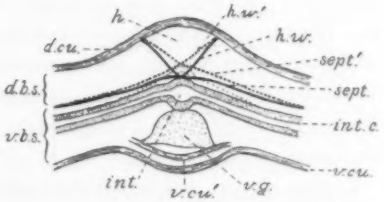

FIG. 2. Diagrammatic cross-section of the body of a pycnogonid through a pair of the lateral processes, illustrating the way in which the heart contracts: *d.b.s.*, dorsal blood space; *d.cu.*, dorsal cuticula; *h.*, lumen of heart; *h.w.*, side wall of heart (at diastole); *h.w.'*, position of side wall of heart at systole; *int.*, main longitudinal trunk of intestine; *int.c.*, intestinal cecum going to leg; *sept.*, transverse horizontal septum (at diastole); *sept.'*, position of transverse septum at systole; *v.b.s.*, ventral blood space; *v.cu.*, cuticula of ventral side, *v.cu.'*, posterior edge of underlapping cuticula of the preceding segment; *v.g.*, ventral ganglion. (Based on the cross-section of a species of *Nymphon* figured by Dohrn, 1881, pl. 15, fig. 10. The ovary, which lies between the intestinal ceca and the transverse septum, has been omitted for the sake of clearness.)

ever, do not completely enclose it, the dorsal wall being formed by the chitinous integument of the back (cf. Fig. 2).

In *Phoxichilidium femoratum* Loman (1907) found that a systole of the heart occurred two or three times each second, which would be 120 to 180 contractions to the minute. A count

of the rate of the heart beat of *Endeis spinosus* which I made at Woods Hole, September 4, 1904, showed 172 contractions per minute, the rate slowing down in a short time after the animal had been mounted under a cover glass. The next morning other specimens showed a rate of 126 to 136 beats per minute. Fig. 1, from a rough sketch made at the time, shows the position and shape of the heart; the dotted lines indicate the apparent change in shape as the muscular side walls contract, and the arrows show the direction of observable streaming of the corpuscles in the body fluid or blood. It must be remembered that owing to the attachment of the side walls to the dorsal integument they must be stationary at this point, the contraction of the muscles in the side walls tending to draw the sides together further down, thus reducing the capacity of the enclosed space (see Fig. 2). It is probable that with the alternate contraction and relaxation of the heart the transverse septum (*sept.*), which divides the body space into dorsal and ventral chambers, is raised (Fig. 2, *sept.*) and lowered to some extent, which would help to force the blood out into the legs at each systole and to draw blood from the legs into the pericardial space at the diastole.

The two pairs of lateral ostia, opposite the lateral processes of the body for the attachment of the second and third pairs of legs respectively, could be plainly distinguished; the single opening at the posterior end was somewhat less distinct. The streaming of the blood cephalad through this portion of the heart left no doubt, however, of the existence of such an opening, except in one case in which the blood could be seen to move back and forth in this region without a definite streaming forward. This would seem to confirm Dohrn's observation that in some cases there is no posterior terminal ostium, or at least it appears that if it existed in this instance it was closed and not functioning.

Although, on the whole, there appeared to be a real circulation from the body out into the legs and back, this was rendered more or less indefinite by the peristaltic contractions of the intestine, which imparted a sort of churning motion to the blood and kept it moving back and forth. This was especially evident in the more expanded femoral joints; in the basal parts of the leg and in the tibia a more definite streaming could be observed. The

contractions of the intestinal ceca in the legs are more or less irregular. There were usually a few (two to four) peristaltic waves in one direction, and then they would change and go the opposite way for an equal time. In some cases the contraction appeared to start at one point and pass from there in both directions along the intestine. In a specimen which had been mounted for some time under a cover glass these peristalses recurred at intervals of two or three seconds.

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THE STRUCTURE OF THE SPERMATOPHORES OF AMBYSTOMA PUNCTATUM.

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Since the recent paper of Wright and Allen ('09) has again directed attention to the breeding habits of *Ambystoma punctatum*, it has occurred to me that a more careful description of the spermatophores than has yet been given might be of value in a comparative survey of the habits of the urodeles. No adequate figures of these spermatophores have yet been published.

The spermatophores of *Ambystoma punctatum* studied by me at Ann Arbor (Smith, '07) were not freshly deposited, and were not in the best possible structural condition for study; hence only a brief description, illustrated by a photograph, was attempted. Since then I have been able to study these structures in abundance under the best possible conditions. As evidence that the spermatophores were freshly deposited, there may be cited the fact that the spermatozoa of some of them were found to be active when examined in the laboratory seven hours after their collection. The results of this study have confirmed the description previously given by me, in regard to distribution and general structure, but have revealed the common occurrence of compound spermatophores, a condition previously unnoticed.

In the early spring of 1909, when the snow had nearly all disappeared, I found specimens of *Ambystoma punctatum* and *A. jeffersonianum*, under rocks on the hillside near "Branchipus Pond," about two miles from the campus of Syracuse University. The study of the habits of these animals was undertaken in order, if possible, to determine by direct observation the exact procedure in the process of fertilization by means of spermatophores in the case of *A. punctatum*, and to obtain data in regard to *A. jeffersonianum*, in which the manner of fertilization is entirely a matter of conjecture.

Conditions made it impossible to keep a very close watch of the pond during the breeding season, and the abruptness of the weather changes caused me to miss the few opportunities for direct

observation of the activites of the animals in the field, by means of a lantern at night. Specimens of both sexes were transported to the laboratory and kept under observation in aquaria; several males of *A. punctatum*, and one of *A. jeffersonianum*, deposited masses of seminal fluid on the floor of the aquarium, but nothing resembling the formation of true spermatophores occurred. The largest of these masses was nearly 2 cm. long, and the quantity of sperm deposited by each individual was amply sufficient to form a group of spermatophores. The behavior of the sexes toward each other presented nothing that could be interpreted as normal breeding behavior.

In the pond large numbers of spermatophores were deposited, and these were carefully studied, both *in situ* and in the laboratory; but all the specimens collected were identified, with considerable certainty, as the product of *A. punctatum*. The failure to solve the main problems caused me to lay my notes and drawings aside, awaiting an opportunity to continue the investigation; but the conclusive results of Wright and Allen ('09) have made this unnecessary so far as regards *A. punctatum*.

Identification of Species.—The fact that *A. jeffersonianum* occurs, though less abundantly, in the same region with *A. punctatum* made necessary a determination of the species of the spermatophores found. The only available method seemed to be through a study of the spermatozoa. The material available consisted of spermatozoa from a considerable number of individuals of *A. punctatum*, taken both at Syracuse and at Ann Arbor, and spermatozoa from one specimen of *A. jeffersonianum*, taken at Syracuse. Material from each individual was fixed by a variety of methods, stained, mounted and carefully measured under high powers of the microscope. The average measurements were as follows:

	Acrosome	Head.	Middle Piece.	Tail.	Total Length.
A. punctatum	20 μ	116 μ	15.0 μ	480 μ	631.0 μ
A. jeffersonianum	17 μ	114 μ	11.3 μ	348 μ	490.3 μ

It will oe seen that the sperm of *A. jeffersonianum* is in general shorter than that of *A. punctatum*. The middle piece is most easily and accurately measured, and affords the readiest means

of differentiating the two species. The range of variation in the length of the middle piece of *A. punctatum* (in several individuals) is 14 μ – 16 μ; in *A. jeffersonianum* (in one individual) 11.2 μ – 11.9 μ.

The fact that only one individual of *A. jeffersonianum* was considered of course militates against the acceptance of these results as absolutely conclusive; but since material was taken from several individuals of *A. punctatum*, and in all of these there was a decided difference from the results obtained from the single individual of *A. jeffersonianum*, it is highly probable that the species may be distinguished in this way.

Spermatozoa were then taken from a dozen or more spermatophores, each from a different group and presumably deposited by a different individual. This material was treated precisely as in the case of the spermatozoa described above. Somewhat to my surprise and disappointment, all the specimens conformed to the dimensions of the sperm of *A. punctatum*.

Structure of the Spermatophores.—Two general types of spermatophores are recognizable: simple and compound. The compound spermatophores may be roughly divided into the vertically serial, and the Y-shaped types, the former by far the most common; but both these conditions may be found combined in one compound spermatophore.

A spermatophore of the simple type is illustrated in Fig. 1. It consists of an expanded hummocky base and a stout stalk, of very clear, transparent, gelatinous material, surmounted by a dome-shaped mass of snowy-white seminal fluid.

While to the naked eye the stalk of a simple spermatophore is perfectly clear, under a low power of the microscope it is seen to possess a delicate network of fibrous material. Under high power this is found to be made up of sparsely distributed spermatozoa, interlaced to form a meshwork. In some spermatophores the strands of this network assume considerable regularity. There may be distinguished regular parallel strands extending in the direction of the axis of the stalk, distributed at about equal intervals through its substance; these strands are connected by more irregular cross-strands.

Evidently a few spermatozoa are present in the cloaca at the

time when the stalk of the spermatophore is laid down, and these spermatozoa owe the regularity of their distribution to the papillated ridges of the cloaca.

For comparison a drawing (Fig. 2) of a spermatophore of *Diëmyctylus viridescens*, obtained from a specimen in captivity, is placed alongside the figure of the simple spermatophore of

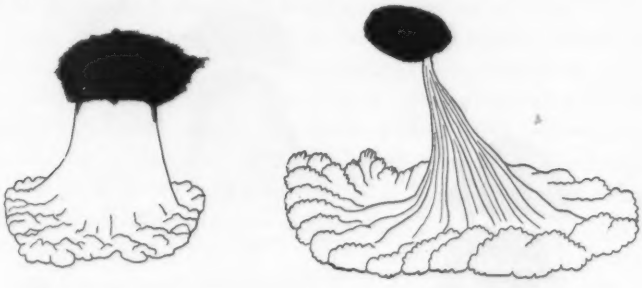

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FIG. 1. Camera drawing of simple spermatophore of *Ambystoma punctatum*, × 5. In this and in the following figures the blackened portion is in nature snowy white.

FIG. 2. Camera drawing of a spermatophore of *Diëmyctylus viridescens*, × 5.

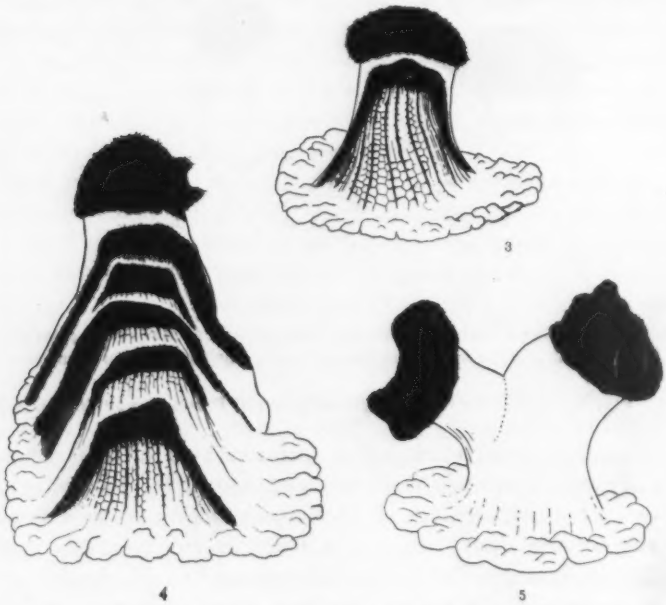
Ambystoma punctatum. (The spermatophore of *Diëmyctylus* has been previously described and figured by Jordan, '93.) Several spermatophores of *Diëmyctylus viridescens* were available for this study, but none of these differed essentially from the one figured. The average dimensions of twelve simple spermatophores of *Ambystoma punctatum* taken at random, and of two uninjured spermatophores of *Diëmyctylus viridescens*, are as follows:

	Total Height.	Longest Diameter of Sperm Mass.	Smallest Diameter of Stalk.	Breadth.
A. punctatum.....	6.2 mm.	3.7 mm.	2.5 mm.	6.4 mm.
D. viridescens.....	8.0 "	2.5 "	0.3 "	12.0 "

The simplest and most common case of compound spermatophore is that in which one spermatophore is deposited directly on top of another (see Fig. 3). In the process of deposition of the second spermatophore the sperm ball of the first one is crushed into the form of an inverted cup covering the stalk of the first, and the sides of this cup are stamped with a characteristic

pattern by the fluted surface of the cloaca. This pattern reminds one of the fainter meshwork seen with the aid of a microscope in the stalk of a simple spermatophore.

By an increase in the number of spermatophores arranged in a vertical series we obtain the extreme form of the serial type shown in Fig. 4, which is made up of six simple spermatophores.



FIGS. 3, 4 and 5. Compound spermatophores of *Ambystoma punctatum*. All the figures are camera drawings. × 5.

FIG. 3. Compound spermatophore consisting of two simple spermatophores, one superimposed on the other.

FIG. 4. Compound spermatophore of the vertical-serial type.

FIG. 5. Simplest case of the Y-shaped type.

Somewhat rarely, compound spermatophores of the Y-shaped type (Fig. 5) are found. These are apparently formed by the deposition of one spermatophore on the side of the stalk of another. In several cases noted, one arm of the Y-shaped stalk was again compound, consisting of two or more spermatophores in series.

Compound spermatophores are not all so regular in form as those figured. Sometimes successive spermatophores are packed together so closely that is it difficult to distinguish individuals in the complex; but while the first impression obtained from some of these spermatophores was that of great irregularity, closer study showed that all of them might be reduced to the vertical-serial type, the Y-shaped type, or a combination of these.

Some of the sperm masses of the simple spermatophores, or the terminal one in the case of a compound spermatophore, were found considerably frayed as if from being taken up into the cloaca of a female; rarely, the entire ball of sperm was missing. It is of course possible that either condition might be accidental.

In one hundred spermatophores taken at random, the number of each kind was as follows:

1. Simple type	23
2. Compound, two in vertical series	45
3. Compound, more than two in vertical series	21
4. Y-shaped, or combination of this type with (2) or (3)	11
Total	100

In none of the spermatophores examined was there any approach to the complexity of structure shown in European urodeles by Zeller ('05). Zeller's drawings appear to me highly conventionalized.

Discussion.—Of the various kinds of spermatophores of *Ambystoma punctatum*, the simple type is functionally the most perfect; yet even this is not so highly differentiated and structurally so well adapted for its purpose as the spermatophore of *Diëmyctylus viridescens*. In case the of *Diëmyctylus*, I have seen almost the entire ball of sperm removed from the very slender stalk by the female cloaca. In *Ambystoma*, on account of the thickness of the stalk affording a large surface for contact with the mass of spermatozoa, this would be more difficult, and according to the observations of Wright and Allen ('09) does not occur—only a very few sperms, comparatively, are detached and retained by the cloaca.

The only mention of compound spermatophores that I find in the literature is in the case of the axolotl, described by Gasco ('81). While in many cases the spermatophores deposited were

single, in one case the male was observed to return repeatedly, at intervals of several minutes, to the same spermatophore and deposit another on top of it, thus building up a compound spermatophore consisting of seven simple spermatophores. Whether the compound spermatophore of *Ambystoma punctatum* is built up in this way I have no means of deciding; it would seem quite as probable that the spermatophores were deposited in rapid succession, while the animal remained in one place — a condition not far removed from what occurs in external fertilization, in which a large amount of seminal fluid is discharged at once. In any case it is difficult to see that any advantage is gained by depositing one spermatophore on top of another and thereby destroying the efficiency of the one previously deposited. As already noted in the case of *Ambystoma punctatum*, the spermatozoa of these spermatophores retain their vitality in water for many hours. The formation of compound spermatophores of the vertical-serial type seems to me a useless and wasteful procedure, and the large proportion of such spermatophores indicates that the spermatophore-forming habit is not highly evolved nor perfectly adapted to its purpose.

In my previous paper (Smith, '07) on the breeding habits of *Ambystoma punctatum*, I ventured the prediction, based on the large number of spermatophores and the manner of their distribution, that the behavior of the adults in the fertilization process would be found to be simpler than in the case of *Diëmyctylus viridescens*. In the latter case only a very few spermatophores are formed by each male, and particular safeguards are necessary in order to insure the delivery of at least one of them to the female cloaca; these safeguards are furnished by the complicated but definite series of acts on the part of the adults which precedes and conditions the deposition of a spermatophore. Just as in the higher vertebrates economy of egg production is correlated with care of the young, so in *Diëmyctylus* economy in the production of spermatophores is accompanied by a considerable amount of certainty as to their fate. This prediction as to the relative simplicity of the behavior of the adults of *Ambystoma punctatum*, the recent observations of Wright and Allen ('09) have abundantly justified. In the light of the observations on

the structure of the spermatophores recorded in the present paper, we can go further and say that the entire process of securing fertilization by means of spermatophores is in *Ambystoma punctatum* more primitive, less highly adapted to secure its object with economy of material, than in *Diëmyctylus viridescens*.

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